

### QIAGEN Midi Plasmid Preps

1. Grow 25-40 ml culture 12-16 hours. Inoculate from a liquid culture that you are sure contains the plasmid you want. Use a 100 ml Erlenmeyer flask that has been previously autoclaved.
2. Transfer the culture to a 50 ml falcon. Spin 10-15 minutes to pellet the cells.
3. Resuspend the cells in 4 ml P1 + RNase by pipetting up and down. Transfer the suspension to a Beckman centrifuge tube that can withstand velocities of up to 20,000 g.
4. Add 4 ml P2 and mix by inverting a few times. Sit 3-5 min. RT, not longer.
5. Add 4 ml P3 and mix by inverting a few times. Incubate on ice 15 minutes.
6. Spin 20,000 g (= 10,000 – 13,000 rpm) at 4 °C, 30 min.
7. Remove the supernatant immediately, and filter this lysate through a whatman paper funnel into a clean 50 ml falcon tube.
8. While step 7 is going on, equilibrate the column (Q-100) by applying 4 ml QBT and letting it flow through by gravity.
9. Load filtered lysate from step 7 onto the column and let it flow through by gravity.
10. Wash twice with about 10 ml QC (fill the column to the top).
11. Transfer the column to a 15 ml corex, and suspend it over the tube with tape (don't let it touch the bottom – leave about 10 ml of space
12. Elute the plasmid DNA into the corex tube with 5 ml QF.
13. Add 3.6 ml isopropanol and mix.
14. Spin 30 min, 4°C, 20,000 g (= 10,000 to 13,000 rpm).
15. Carry a beaker to the centrifuge with you when you are ready to remove the tubes, and discard the supernatant immediately, so that the pellet does not have time to dislodge from the side of the tube. Remember that isopropanol pellets are clear and thin, do not expect to see a huge pellet.
16. Wash with 2 ml 70% EtOH, and spin again, 30 min, 4°C, 20,000 g (= 10,000 – 13,000 rpm).
17. Discard the EtOH and dry the pellet. Air-drying takes forever, so either use a hair dryer or pull a Pasteur pipette to a thin point and use that to dry the pellet.
18. Resuspend DNA in TE or water: expect about 100 ug DNA, so resuspend in 100 ul for 1 ug/ml, or in 1 ml for 100 ng/ml.